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Joint Action of O3 and SO2 in Modifying Plant Gas Exchange

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David M. Olszyk and David T. Tingey

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16. ABSTRACT

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Joint Action of O_3 and SO_2 in Modifying Plant Gas Exchange[1]

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DAVID M. OLSZYK*[2] AND DAVID T. TINGEY
Statewide Air Pollution Research Center, University of California, Riverside, Riverside, California 92521 (D.M.O.); *and United States Environmental Protection Agency, Corvallis, Oregon 97333* (D.T.T.)

ABSTRACT

The joint action of O_3 and SO_2 stress on plants was investigated by determining the quantitative relationship between air pollutant fluxes and effects on stomatal conductance. Gas exchange measurements of O_3, SO_2, and H_2O vapor were made for *Pisum sativum* L. (garden pea). Plants were grown under controlled environments, and O_3, SO_2, and H_2O vapor fluxes were evaluated with a whole-plant gas exchange chamber using the mass-balance approach. Maximum O_3 and SO_2 fluxes per unit area (2 sided) into leaves averaged 8 nanomoles per square meter per second with exposure to either O_3 or SO_2 at 0.1 microliters per liter. Internal fluxes of either O_3 or SO_2 were reduced by up to 50% during exposure to combined *versus* individual pollutants; the greatest reduction occurred with simultaneous *versus* sequential combinations of the pollutants. Stomatal conductance to H_2O was substantially altered by the pollutant exposures, with O_3 molecules twice as effective as SO_2 molecules in inducing stomatal closure. Stomatal conductance was related to the integrated dose of pollutants. The regression equations relating integrated dose to stomatal conductance were similar with O_3 alone, O_3 plus added SO_2, and O_3 plus SO_2 simultaneously; *i.e.* a dose of 100 micromoles per square meter produced a 39 to 45% reduction in conductance over nonexposed plants. With SO_2 alone, or SO_2 plus added O_3, a dose of 100 micromoles per square meter produced a 20 to 25% reduction in conductance. When O_3 was present at the start of the exposure, then stomatal response resembled that for O_3 more than the response for SO_2. This study indicated that stomatal responses with combinations of O_3 and SO_2 are not dependent solely on the integrated dose of pollutants, but suggests that a metabolic synergistic effect exists.

A crucial question in interpreting the nature of the joint action response between O_3 and SO_2 stresses is the relationship between the amount of pollutant dose and the magnitude of the response. Previous studies have described effects on the basis of pollutant concentrations in the ambient air surrounding the plant (1, 2, 4, 7, 11). None of the studies considered the actual absorbed dose of both pollutants to the leaves. Yet pollutant dose is a key factor in using toxicological models that test for an interaction (6, 11). These models indicate that a true synergistic effect occurs only if the response from the dose of both gases is greater than the response anticipated from additive effects of the individual gas doses. To date, interaction studies have not been designed to test this toxicological model, even though the results could yield information on the physiological mechanism for joint action of combinations of pollutants (11).

A plant response common to O_3 and SO_2 exposures is required to test the joint action model. Decreases in stomatal conductance have been reported for plants exposed to O_3 or SO_2 singly or in combination (1, 5, 7, 12). The decreases in conductance were often greater with combined exposures, suggesting pollutant interaction. Conductance is an especially amenable indicator of immediate responses and can be accurately measured during pollutant exposures (12). It also is an important physiological indicator of air pollutant stress since it is a key process in subsequent injury, growth, and yield effects from pollutants.

This study was designed to characterize the important physiological events correlated with exposure to combinations of O_3 plus SO_2, by determining the relationship between integrated pollutant dose and stomatal conductance.

MATERIALS AND METHODS

Plant Culture. Peas (*Pisum sativum* L. cv Alsweet) were grown and exposed using published procedures detailed previously (8, 10). Peas had exhibited consistent synergistic injury and stomatal conductance responses in experiments to evaluate the joint action of O_3 and SO_2 in plants (7, 11). Plants were grown from seed in an artificial medium (Promix BX[2]),[3] and watered with North Carolina State University nutrient solution. They were cultured in controlled environment growth chambers with light/dark air temperature of approximately 20 ± 1°C, PPFD[4] of 301 ± 18 μmol m^{-2} s^{-1} for 16 h/24 h photoperiod, and light/dark RH of 76/90. Gas exchange was measured when peas had six fully expanded leaves at 19 to 21 d after seeding. Pea leaf area (2-surface) averaged 0.033 ± 0.001 (SE) m^2.

Gas Exchange System. Fluxes of O_3, SO_2, and H_2O vapor were measured using a whole-plant gas exchange chamber (8, 15). Environmental conditions were controlled in the chamber at approximately 300 μmol m^{-2} s^{-1} PPFD 21°C air temperature and 16°C dew point temperature to provide 70% RH and 370 μl L^{-1} CO_2, using procedures described earlier (9).

Air dewpoint was maintained by manipulating the amount of water vapor in the incoming air stream. Water vapor was initially removed from the air stream with a Drierite (Hammond Anhydrous) filter, and then added at a controlled rate by passing the air through a bubbler system. Dewpoint was measured using a hygrometer (ElectroMech CTE 84 DR) calibrated at 0°C over ice.

CO_2 was scrubbed from the incoming air stream with an Ascarite II (Arthur H. Thomas Co.) filter after the water vapor was removed. CO_2 was then metered back into the air stream from a tank of pure CO_2 at a controlled rate adequate to maintain the desired concentration in the gas exchange chamber. CO_2 was monitored with IR analyzers (Mine Safety Appliances and Beckman model 865) and checked daily with pure N_2 (0 CO_2) and

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[3] Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

[4] Abbreviation: PPFD, photosynthetic photon flux density.

standard calibration gas cylinders ranging from 350 to 400 $\mu l\ L^{-1}\ CO_2$.

Sulfur dioxide from a permeation tube housed in a constant-temperature oven was metered into the incoming air stream. It was monitored with flame photometric analyzers (Meloy models 160 and 185) equipped with H_2S scrubbers. Ozone, generated by irradiating a portion of the incoming air stream with UV light, was monitored with UV absorption analyzers (Dasibi model 1003AH). Daily zero and span checks for analyzers were made using a Monitor Laboratories model 8500 Calibrator. Both SO_2 and O_3 analyzers had routine multipoint calibrations with transfer standards *i.e.* a Bendix model 8861 DATM Field Calibration System for SO_2 and a Dasibi model 1008PC O_3 analyzer for O_3. Pollutant, CO_2, and H_2O vapor concentrations were measured at chamber inlet and outlet.

The daily schedule for gas exchange experiments began by placing plants in the gas exchange system at 1700 the day before measurement. The chamber was left open to prevent CO_2 buildup during the 0200 to 1000 dark period. The chamber was sealed at 0600 and the gas exchange system was turned on. Single O_3 or SO_2, or simultaneous O_3 + SO_2, exposures began at 0800 and were terminated just prior to beginning of the light period. Exposures were again initiated at 1200, 2 h after the start of the light period, and continued for 4 or 5 h. For the sequential combination exposures, O_3 or SO_2 was added between 0900 and 1000 in the dark, and between 1420 and 1700 in the light. At approximately 1700 a plant was harvested and projected (one sided) leaf area was measured (Hayashi Denko model AAM-5 and LI COR® model 3100 leaf area meters).

Chamber Loss Measurements. The inlet-outlet differences in O_3, SO_2, and H_2O vapor concentration were adjusted for flux to the chamber system by running the system at the identical temperature, dewpoint, and light intensity as when a plant was present for the same duration as plant exposures (8, 10). Flux of SO_2 into the chamber decreased exponentially over time during both the dark and subsequent light period and was not affected by the presence of O_3 in the system. The flux ranged from 0.2 nmol s^{-1} after 1 h in the dark to 0.08 nmol s^{-1} after 5 h in the light. Flux of H_2O vapor into the chamber was a constant 2.5 mmol s^{-1} in both dark and light over the exposure period and was unaffected by addition of O_3 and/or SO_2 to the system (10).

Flux of O_3 into the chamber varied slighty with different conditions. In the dark with or without added SO_2, flux of O_3 at a concentration of 0.11 $\mu l\ L^{-1}$ O_3 was approximately 0.2 nmol s^{-1}. In the light with 0.11 $\mu l\ L^{-1}$, O_3 loss was initially 0.1 to 0.15 nmol s^{-1}, declining by approximately 15% after 4 h. There was a slight variability in O_3 loss depending on the presence or absence of added SO_2 in the chamber. Chamber fluxes for O_3 and SO_2 were the same magnitude as the fluxes to plants, *i.e.* 0.1 to 0.2 nmol s^{-1}.

Leaf Surface Fluxes. Previous research indicated that pea stomata were not fully closed in the dark (10). Therefore, to obtain estimates of pollutant fluxes to leaf surfaces, plants were sprayed until runoff with 0.1 mmol ABA using an air brush (Badger 250) with nitrogen propellant to enhance stomatal closure at night. Plants were sprayed in the dark just prior to placing plants in the gas exchange chamber. The ABA was dissolved in distilled H_2O vapor with one or two drops of Triton X-100 added per 50 ml of solution. Flux measurements were initiated after all liquid had evaporated from leaves.

Fluxes of pollutants to pea leaf surfaces averaged 2.9 ± 0.5 (SE) nmol $m^{-2}\ s^{-1}$ for O_3 and 1.9 ± 1.0 nmol $m^{-2}\ s^{-1}$ for SO_2, as determined from four single plant replicates exposed to a combination of 0.11 $\mu l\ L^{-1}$ O_3 plus 0.12 $\mu l\ L^{-1}$ SO_2. There also was a flux of 0.32 ± 0.07 mmol $m^{-2}\ s^{-1}$ of H_2O vapor from leaf surfaces that was subtracted from the total H_2O vapor loss from leaves in the light in order to determine stomatal conductance. Few published data exist for surface flux rates of O_3, SO_2, and H_2O, for plants exposed to concentrations of O_3 or SO_2 as low as 0.1 $\mu l\ L^{-1}$. Previously reported SO_2 and H_2O surface flux rates were lower for peas than for several other species (10).

Flux and Conductance Calculations. Fluxes of O_3, SO_2, and H_2O vapor, and stomatal conductances (10) of H_2O, were determined according to formulas described previously. All gas exchange rates were calculated on a two-surface basis by doubling the projected leaf area. Boundary layer resistance was assumed to be a low value of 0.10 cm^{-1} as there was leaf flutter in the gas exchange system due to mixing of the air via fans, and because no boundary layer resistance could be detected using the Sestak *et al.* (13) energy balance method of determining boundary layer resistance.

Only the flux in the light was used for comparison to stomatal response. The stomata were open slightly in the dark with an average conductance of 0.087 ± 0.024 cm s^{-1} over all the treatments. There was a small flux in the dark; average of 2.3 and 1.1 nmol $m^{-2}\ s^{-1}$, respectively, for O_3 and SO_2. The O_3 and SO_2 fluxes in dark were not considered in the calculations of integrated pollutant dose as they were quite small compared to flux in the light, and because the plants had 2 h in the light without exposure to recover from any effects of pollutants before being exposed again.

Statistical Analysis. Four plants were used to determine gas exchange for each pollutant treatment, thus providing four single plant replicates per measurement. Two-way analysis of variance with time during exposure and replicate plants as factors were used to determine standard errors for fluxes or stomatal conductance (14). One-tailed *t* tests were used for comparing means. Best fit regression analysis was used to compare total pollutant absorption to stomatal conductance. The analysis considered linear, exponential, and hyperbolic curves.

RESULTS AND DISCUSSION

Pollutant Absorption. As previous studies (3) have shown, peas exposed to combinations of O_3 and SO_2 displayed large decreases in pollutant flux compared with plants exposed to the single gases. Exposure to O_3 alone resulted in a peak O_3 flux after 1 h, followed by a 25% decrease after 5 h (Fig. 1A). Flux for plants exposed to SO_2 alone increased gradually over the 5 h exposure (Fig. 1B). Addition of either SO_2 (Fig. 1C) or O_3 (Fig. 1D) to an ongoing exposure resulted in dramatic decreases in fluxes for both pollutants. Two h after the second pollutant was added, the fluxes of both pollutants decreased by 35 to 50% compared with the fluxes 1 h after addition of the second pollutant.

Plants treated to O_3 plus SO_2 simultaneously from the beginning of the exposure had initial O_3 flux rates slightly lower, and SO_2 fluxes slightly higher, than with the single pollutants (Fig. 1E). The fluxes for both pollutants decreased gradually, *i.e.* 38% after 4 h of exposure.

The integrated doses of O_3 and/or SO_2 over time increased for all treatments (Fig. 2). The dose was greater with the combinations than with the single gas exposures, reflecting the extra pollutant available for uptake when both pollutants were present simultaneously for the entire period. After 4 h, 100 $\mu mol\ m^{-2}$ of gas were absorbed for O_3 alone (Fig. 2A), SO_2 alone (Fig. 2B), and SO_2 plus added O_3 (Fig. 2D). After the same period, 135 $\mu mol\ m^{-2}$ of gas were absorbed with O_3 plus added SO_2 (Fig. 2C), while over 140 $\mu mol\ m^{-2}$ was absorbed with O_3 plus SO_2 simultaneously (Fig. 2E).

Stomatal Conductance. Data from control plants indicated that stomatal conductance increased gradually over the entire period that the plants were in the chamber (Fig. 3A). The increasing stomatal conductance may have been related to the relative low PPFD in the exposure chamber or diurnal variation in conductance under these exposure conditions. Unfortunately,

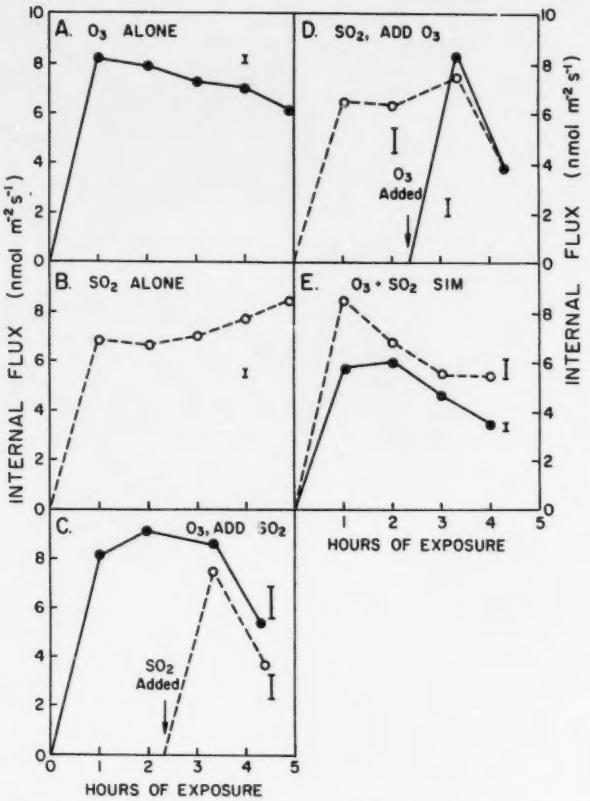

FIG. 1. Internal pollutant flux for (A) O_3 alone, (B) SO_2 alone, (C) O_3 plus added SO_2, (D) SO_2 plus added O_3, and (E) O_3 plus SO_2 simultaneously. The O_3 concentrations averaged 0.11 μl L^{-1} and SO_2 concentrations averaged 0.12 μl L^{-1}. The bars equal 1 SE averaged across all hours of exposure for a treatment. (●——●), (○- - -○), Averages for four single plant replicates for O_3 and SO_2. Internal pollutant flux was determined as $J_{Internal}$ (nmol m^{-2} s^{-1}) = [[[C (nmol m^{-2} s^{-1}) × F (m^3 s^{-1})] − U (nmol s^{-1})] × A^{-1}(m^2)] − $J_{Surface}$ where C = the concentration of SO_2 or O_3 as it passed through the chamber; F = air flow rate through the chamber; U = flux of SO_2 or O_3 to the chamber; and A = leaf area (2 surfaces).

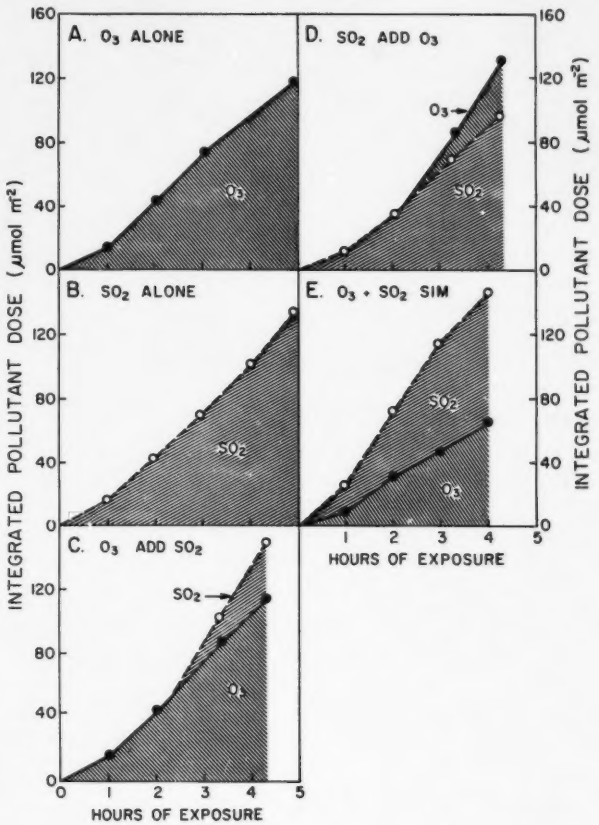

FIG. 2. Integrated pollutant dose ascribed to O_3 (▨) or SO_2 (▨), with exposures to (A) O_3 alone, (B) SO_2 alone, (C) O_3 plus added SO_2, (D) SO_2 plus added O_3, and (E) O_3 plus SO_2 simultaneously. The O_3 concentrations averaged 0.11 μl L^{-1}, and SO_2 concentrations averaged 0.12 μl L^{-1}. (●) or (○), means for four single plant replicates. Integrated dose (D) (μmol m^{-2}) was determined as D = sum (mean pollutant flux per each hour of exposure [nmol m^{-2} s^{-1}] × 3.6 × 10^3 s h^{-1}) over all hours of exposure.

gas exchange measurements were never continued for longer than 7 h into the 16 h light period and the maximum stomatal conductance may have been undetected. However, each group of treatment plants had different initial and peak stomatal conductances in the light, even though they had similar patterns of increased conductance over time. Thus, the control plants could not be reliably used to indicate the reference conductance that would have occurred at different hours during the exposures if plants had not been treated with air pollutants, for comparison with the exposure conductances.

The trend in stomatal conductance during the 2 h in the light before the exposures began was used to predict the conductance that would have occurred through h 5 for each group of plants if there had been no pollutant treatment. The predicted conductance was then used as the reference for determining the pollutant-induced stomatal response which was quantified by calculating the exposure conductance as a percentage of the predicted conductance for each h of measurement.

The validity of this prediction was evaluated with data from the nonexposed 'control' plants. For these plants, the regression equation for the −1 and 0 time periods was stomatal conductance = 0.2197 × h in light$^{0.2713}$, with n = 8, and r = 0.588 (Fig. 3A). This equation is similar to the equation based on all seven time periods, *i.e.* stomatal conductance = 0.2228 × h in light$^{0.2449}$, with n = 28, and r = 0.924. Exponential conductance equations seemed to be reasonable for the prediction as they accurately predicted conductance values after 1 h of exposure for all treatments except SO_2 plus added O_3, in which case the early SO_2 treatment may have stimulated an initial increase in conductance.

As reported for other species (1, 2, 5, 7), stomatal conductance was reduced with all exposures to O_3 and SO_2 alone or in combination over a several hours exposure period. The treatments differed in their ability to reduce conductance in the following sequence: O_3 plus SO_2 simultaneously > O_3 plus added SO_2 > O_3 alone > SO_2 plus added O_3 > SO_2 alone. Stomatal conductance began to decrease after 2 h with O_3 alone (Fig. 3B), SO_2 alone (Fig. 3C), and O_3 plus SO_2 simultaneously (Fig. 3F). Conductance decreased slightly after 2 h exposure to O_3, and then decreased dramatically after addition of SO_2 to the ongoing O_3 exposure (Fig. 3D). Conductance apparently increased dramatically after 1- and 2-h exposure with SO_2, and then decreased dramatically after addition of O_3 to the ongoing SO_2 exposure (Fig. 3E). Additional research is needed to determine whether the increase in conductance was real or whether it may have

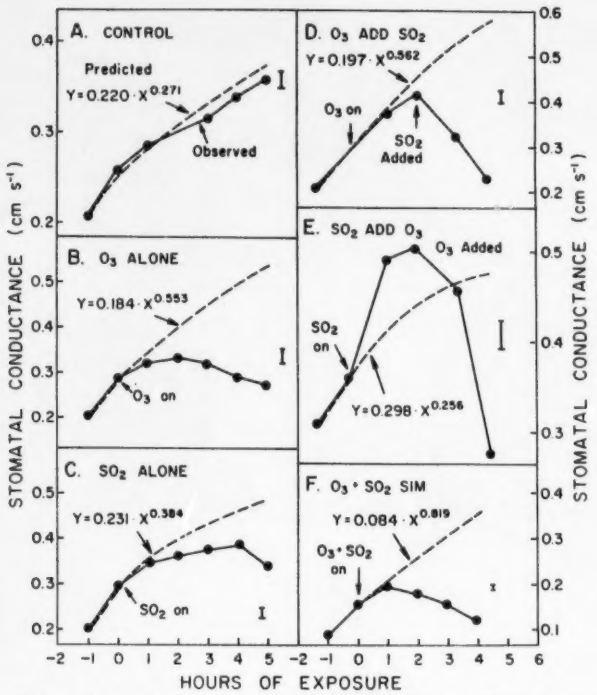

FIG. 3. Stomatal conductance for (A) control plants, (B) O_3 alone, (C) SO_2 alone, (D) O_3 plus added SO_2, (E) SO_2 plus added O_3, and (F) O_3 plus SO_2 simultaneously. The O_3 concentrations averaged 0.11 μl L^{-1}, and SO_2 concentrations averaged 0.12 μl L^{-1}. The bars equal 1 SE for all hours across a treatment. The symbols (●) are means for four single plant replicates. The dashed lines (-----) represent predicted stomatal conductances based on exponential equations calculated from the −1 and 0 h data for each treatment. Conductance was determined as g_s (cm s^{-1}) = (Tr [mmol m^{-2} s^{-1}] × C^{-1} [mmol m^{-3}] × 10^2 cm m^{-1}) − 0.10 cm s^{-1} boundary layer conductance, where Tr = flux of H_2O vapor from inside leaves (2 surfaces); and C = the change in concentration of H_2O vapor between leaf interior and ambient air assuming 100% humidity inside leaves and considering air and leaf temperatures.

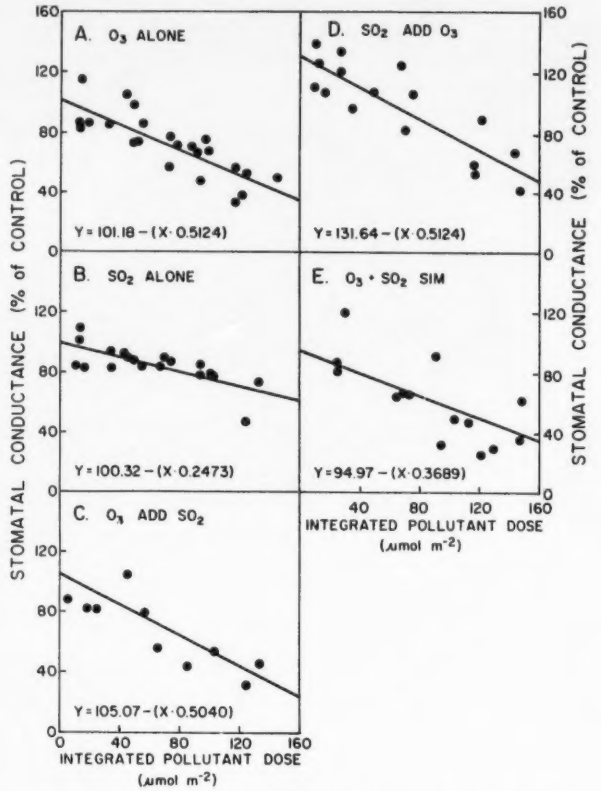

FIG. 4. Integrated pollutant dose *versus* stomatal conductance (percent of predicted control) for different pollutant treatments. Each symbol (●) represents data from one plant. Regression equations are shown for each treatment, other statistical parameters are: (A) O_3 alone, $r = 0.8136$, $s_{yx} = 11.9169$, and $n = 20$; (B) SO_2 alone, $r = 0.7506$, $S_{yx} = 8.3950$, and $n = 20$; (C) O_3 plus added SO_2, $r = 0.8104$, $S_{yx} = 16.5030$, and $n = 14$; (D) SO_2 plus added O_3, $r = 0.8496$, $S_{yx} = 16.3874$, and $n = 16$; and (E) O_3 plus SO_2 simultaneously, $r = 0.7179$, $S_{yx} = 19.1311$, and $n = 16$.

been an artifact since it was not found with SO_2 alone.

Integrated Pollutant Dose *versus* Stomatal Conductance. Linear equations best described the relationships between integrated pollutant absorbed during the exposure *versus* percent decrease in stomatal conductance from the predicted values. The equations were most similar for O_3 alone (Fig. 4A), O_3 plus added SO_2 (Fig. 4C), and SO_2 plus added O_3 (Fig. 4D). All three equations had slopes greater than 0.50 and correlation coefficients greater than 0.81. The y intercept for the SO_2 plus added O_3 equation was greater than 100, reflecting the possible stimulation of stomatal opening by SO_2 during the initial part of this combination exposure. The equation for O_3 plus SO_2 simultaneously (Fig. 4E), resembled O_3 alone and O_3 plus added SO_2 in terms of the y intercept; however, the slope was not as steep. For SO_2 alone, the relationship between dose and decrease in stomatal conductance was different from the other treatments (Fig. 4B). The y intercept was 100 but the slope was much less steep.

Ozone produced almost twice as much stomatal closure as SO_2 per unit dose. For example, stomatal conductance was reduced by 39% from the control level when 100 μmol m^{-2} of O_3 was absorbed, compared to a 24% reduction when 100 μmol m^{-2} of SO_2 was absorbed. Thus, stomatal conductance was reduced by approximately 0.39% and 0.24%, respectively, for each μmol m^{-2} of O_3 or SO_2 absorbed. These conductance *versus* dose ratios

Table I. *Integrated Pollutant Dose Versus Expected and Actual Decreases in Stomatal Conductance for Peas Exposed to Combinations of O_3 and SO_2*

Total integrated pollutant dose and the portions ascribed to O_3 and SO_2 are based on Figure 2. Expected stomatal conductance is based on the sum of (O_3 dose in μmol m^{-2} × 0.39% reduction in conductance per μmol m^{-2}) + (SO_2 dose in μmol m^{-2} × 0.24% reduction in conductance per μmol m^{-2}). Actual stomatal conductances ± SD (from S_{yx}) were determined according to the equations given for Figure 4.

Treatment	Pollutant Dose			Stomatal Conductance	
	Total	O_3	SO_2	Expected	Actual
	μmol m⁻²				*% predicted*
O_3, add SO_2	80	70	10	71	65 ± 17
	100	84	16	64	55 ± 17
	120	96	24	57	45 ± 17
SO_2, add O_3	80	12	68	80	91 ± 16
	100	20	80	73	80 ± 16
	120	28	92	68	70 ± 16
O_3 + SO_2 simultaneously	80	34	46	77	66 ± 10
	100	41	59	71	58 ± 10
	120	50	70	64	51 ± 10

were used to determine the stomatal conductance expected with different doses for the combination treatments, compared with the actual stomatal conductance measured for those treatments (Table I).

The actual stomatal conductance was less than that expected for plants exposed to combinations with a long period of exposure to O_3, *i.e.* O_3 with added SO_2, and O_3 plus SO_2 simultaneously (Table I). Thus, there may have been a true 'synergistic' effect, with greater reduction in stomatal conductance than predicted from the effects of O_3 and SO_2 molecules alone. The converse may have been true with the SO_2 plus added O_3 exposures, *i.e.* there was an apparent 'antagonistic' effect with low pollutant absorption. However, the importance of the expected *versus* actual comparisons is uncertain as the expected values still generally fell within the standard deviations around the regression lines shown in Figure 4. Furthermore, the O_3 'antagonism' is questionable because of the large increase in conductance found with SO_2 before O_3 was added. The conductance coefficient for SO_2 (0.24% reduction in conductance per μmol m^{-2} SO_2) may not be applicable for this treatment.

Evaluation of Interaction Responses. The physiological basis for joint action by O_3 plus SO_2 in producing injury to plants remains intriguing. The relatively low light intensity used in this study may limit generalization from the results. However, the data suggested that even though the greater stomatal closure with combined *versus* single pollutants can be in part attributed to greater pollutant dose, there is additional stomatal closure reflecting a synergistic response between O_3 and SO_2. The metabolic basis for synergistic injury between O_3 and SO_2 in plants likely involves biochemical events within leaf cells which increase the toxicity of O_3 and/or SO_2 molecules (8, 9). However, to accurately test whether a key biochemical response is involved with the synergistic effect, *e.g.* integrity of cell membrane components, any mechanistic study would have to consider the dose of O_3 and SO_2 during the pollutant treatment.

On the whole, these results reinforce the previously documented response that low levels of O_3 plus SO_2 cause reductions in fluxes of either pollutant, even though the sum of the fluxes (O_3 plus SO_2) was greater than for either pollutant alone. The data demonstrate a likely direct relationship between decreased stomatal conductance and the decreased fluxes. Earlier studies had indicated both the flux (3) and conductance reductions (2), but had not conducted simultaneous measurements of both parameters. As a result, it has not been possible to determine whether or not the decreases in pollutant flux were associated with lowering of a 'residual resistance' to O_3 and SO_2 flux in addition to a change in stomatal conductance (16). Because the changes in conductance for O_3 and SO_2 closely paralleled reductions in pollutant absorption, the pea data reported have suggested that pollutant flux is not greatly affected by any residual resistance.

CONCLUSIONS

Ozone, SO_2, or combinations of both gases all decreased stomatal conductance in short-term, low pollutant concentration exposures. The decreases in conductance from predicted values were related to the integrated pollutant dose absorbed over time. Ozone molecules had a greater effect in reducing stomatal conductance than SO_2 molecules. A synergistic effect was suggested with the O_3 plus added SO_2, and simultaneous O_3 plus SO_2 exposures, as there was more stomatal closure than expected by the dose with combined *versus* single pollutants exposures. Additional experiments are needed to identify biochemical events within leaves which affect the joint toxicity of O_3 and SO_2.

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LITERATURE CITED

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